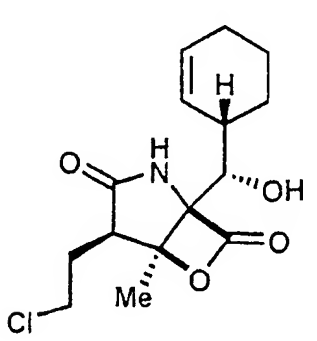
PATENT APPLICATION
Docket No. 04979.00045

SIMPLE STEREOCONTROLLED SYNTHESIS OF SALINOSPORAMIDE A

BACKGROUND OF THE INVENTION

Salinosporamide A **(1)** was recently discovered by Fenical et al. as a bioactive product of a marine microorganism that is widely distributed in ocean sediments. Feeling, R.H.; Buchanan, G.O.; Mincer, T.J.; Kauffman, C.A.; Jensen, P.R.; Fenical, W., *Angew. Chem. Int. Ed.*, 2003, 42, 355-357.



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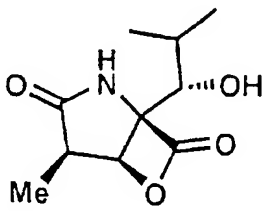
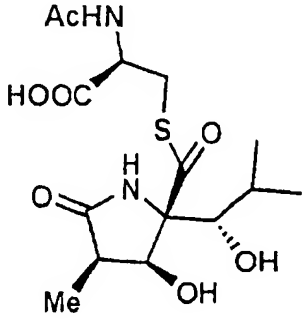


2a



2b

Structurally Salinosporamide A closely resembles the terrestrial microbial product omuralide (**2a**) that was synthesized by Corey et al. several years ago and demonstrated to be a potent inhibitor of proteasome function. See, (a) Corey, E.J.; Li, W. D., *Z. Chem.*

Pharm. Bull., 1999, 47, 1-10; (b) Corey, E.J., Reichard, G.A.; Kania, R., *Tetrahedron Lett.*,1993, 34, 6977-6980; (c) Corey, E. J.; Reichard, G. A., *J. Am. Chem. Soc.*, 1992, 114, 10677-10678; (d) Fenteany, G.; Standaert, R.F.; Reichard, G. A.; Corey, E. J.; Schreiber, S. L., *Proc. Natl. Acad. Sci. USA*, 1994, 91, 3358-3362.

Omuralide is generated by β-lactonization of the N-acetylcysteine thiolester lactacystin (**2b**) that was first isolated by the Omura group as a result of microbial screening for nerve growth factor-like activity. See, Omura, S., Fujimoto, T., Otoguro, K., Matsuzaki, K., Moriguchi, R., Tanaka, H., Sasaki, Y., *Antibiot.*, 1991, 44, 113-116; Omura, S., Matsuzaki, K., Fujimoto, T., Kosuge, K., Furuya, T., Fujita, S., Nakagawa, A., *J. Antibiot.*, 1991, 44, 117-118.

Salinosporamide A, the first compound Fenical's group isolated from *Salinospora*, not only had a never-before-seen chemical structure **1**, but is also a highly selective and potent inhibitor of cancer-cell growth. The compound is an even more effective proteasome inhibitor than omuralide and, in addition, it displays surprisingly high *in vitro* cytotoxic activity against many tumor cell lines (IC_{50} values of 10 nM or less). Fenical et al. first found the microbe, which they've dubbed *Salinospora*, off the coasts of the Bahamas and in the Red Sea. See, *Appl. Environ. Microbiol.*, 68, 5005 (2002).

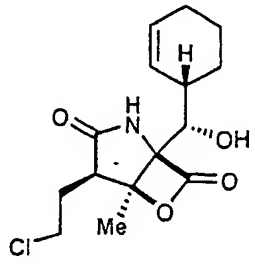
Fenical et al. have shown that *Salinospora* species requires a salt environment to live. *Salinospora* thrives in hostile ocean-bottom conditions: no light, low temperature, and high pressure. The Fenical group has now identified *Salinospora* in five oceans, and with 10,000 organisms per cm^3 of sediment and several distinct strains in each sample; and according to press reports, they've been able to isolate 5,000 strains. See, *Chemical & Engineering News*, 81, 37 (2003).

A great percentage of the cultures Fenical et al. have tested are said to have shown both anticancer and antibiotic activity. Like omuralide **2a**, salinosporamide A inhibits the proteasome, an intracellular enzyme complex that destroys proteins the cell no longer

needs. Without the proteasome, proteins would build up and clog cellular machinery. Fast-growing cancer cells make especially heavy use of the proteasome, so thwarting its action is a compelling drug strategy. See, Fenical et al., U.S. Patent Publication No. 2003-0157695A1, the disclosure of which is hereby incorporated herein by reference.

SUMMARY OF THE INVENTION

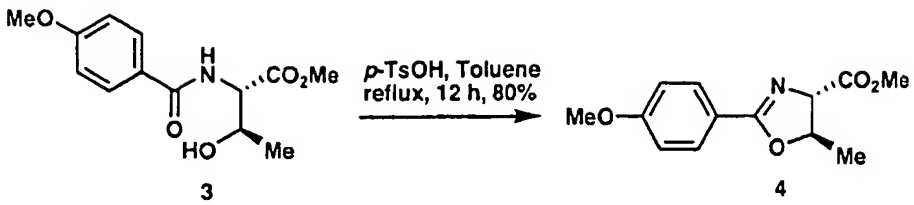
The present invention is directed to a method for the enantiospecific total synthesis of the compound of structure **1**.



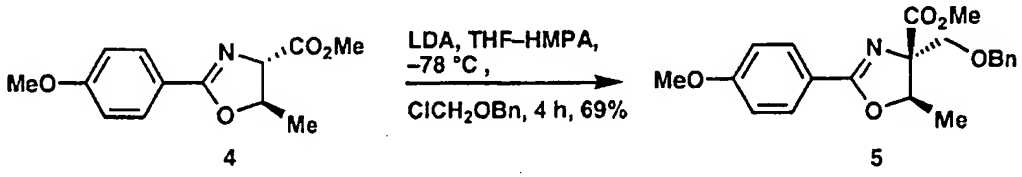
1

The preferred synthetic route to compound **1** is illustrated in the Figure accompanying this specification, and as discussed in greater detail below. In summary, the method of the present invention includes the following steps, which are detailed here with the preferred reagents and reaction conditions. The skilled artisan may likely be able to make substitutions of reagents and/or reaction conditions in any one or more of these reaction steps without necessarily departing from scope and teachings of the present invention:

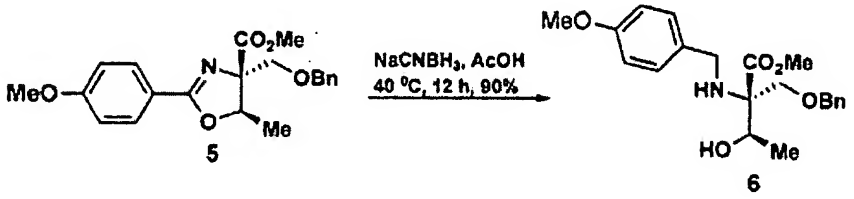
(S)-Threonine methyl ester was N-acylated with 4-methoxybenzoly chloride in CH_2Cl_2 at 23°C to form the amide **3** (71%) which was then cyclized to oxazoline **4** (80%) by heating at reflux in toluene with p-toluenesulfonic acid.



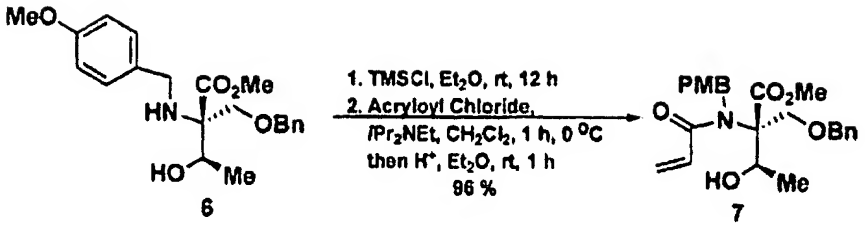
Deprotonation of **4** with lithium diisopropylamide in THF and alkylation of the resulting enolate with chloromethyl benzyl ether afforded the required tertiary stereocenter of **5** selectively in 69% yield.



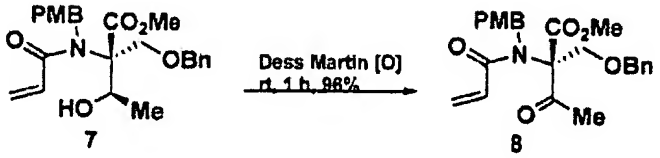
Reduction of **5** with $NaBH_3CN$-HOAc gave the N-4-methoxybenzylamine **6** (90%).



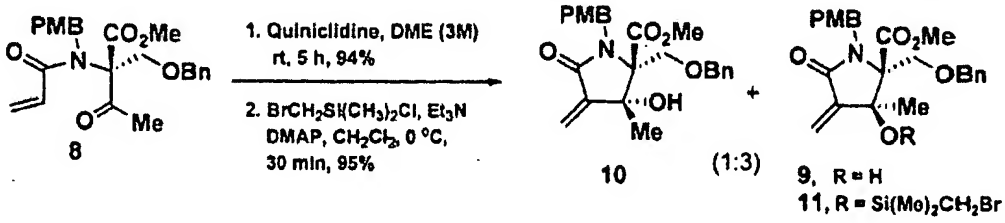
Compound **6** was then transformed in 96% yield to the N-acrylyl-N-PMB derivative **7** (PMB = 4-methoxybenzyl) by the one flask, seqeuence: (1) reaction with Me_3SiCl and Et_3N to form the TMS ether (**6a** - OH is OTMS), followed by (2) acylation with acrylyl chloride at 0°C and (3) acidic work up with aqueous HCl.



Dess-Martin periodinane oxidation of 7 produced the keto amide ester **8** in 96% yield.



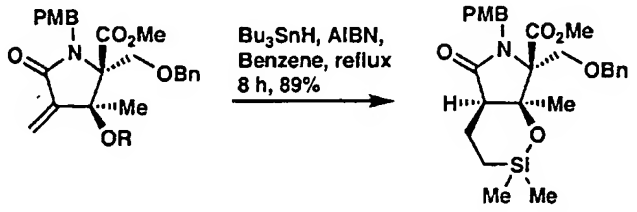
Cyclization of **8** to the γ-lactam **9** was accomplished by means of an internal Baylis-Hillman-aldol reaction using quinuclidine as the catalytic base in dimethoxyethane at 23°C for 5 h. See, Frank, S. A.; Mergott, D. J., Roush, W. R., *J. Am. Chem. Soc.*, 2002, 124, 2404-2405. Mergott, D.J., Frank, S. A., Roush, W. R., *Org. Lett.*, 2002, 4, 3157-3160. Aggarwal, V. K., Emme, I., Fulford, S. Y., *J. Org. Chem.*, 2003, 68, 692-700. Yeo, J. E., Yang, X., Kim, H.J., Koo, S., *J. Chem. Soc., Chem. Commun.*, 2004, 236-237. The cyclization product, obtained in 94% yield consisted of **9** and the C(β) diastereomer (**10**) in a ratio of 3:1.



The N-benzyl analog of **10** (not shown) was obtained in crystalline form mp = 136-7°C, and demonstrated to possess the stereochemistry shown by single crystal X-ray diffraction analysis.

Silylation of **9** with bromomethyldimethylsilyl chloride afforded **11** in 95% yield. Silyl ether **11** and the diastereomeric silyl ether were easily and conveniently separated at this stage by silica gel column chromatography on a preparative scale.

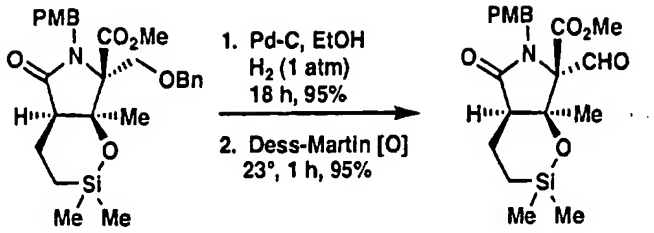
The required stereochemical relationship about C(α) and C(β) of the γ-lactam core was established by tri-*n*-butyltin hydride-mediated radical-chain cyclization which transformed **11** cleanly into the *cis*-fused γ-lactam **12**.



11 **12**

See, (a) Bols, M., Skrydstrup, T., *Chem. Rev.,* 1995, 95, 1253-1277. (b) Fleming, I., Barbero, A., Walter, D., *Chem. Rev.,* 1997, 97, 2063-2092. (c) Stork, G., Mook, R., Biller, S.A., Rychnovsky, S. D., *J. Am. Chem. Soc.,* 1983, 105, 3741-3742. (d) Stork, G., Sher, P. M., Chen, H.L., *J. Am. Chem. Soc.,* 1986, 108, 6384-6385.

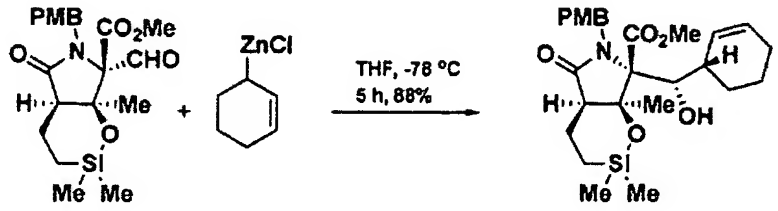
Cleavage of the benzyl ether of **12** (H_2, Pd-C) afforded the primary alcohol (**12a** – OBn is OH), and Dess-Martin periodinane oxidation of **12a** provided the aldehyde **13** in about 90% yield from **12**.



12 13

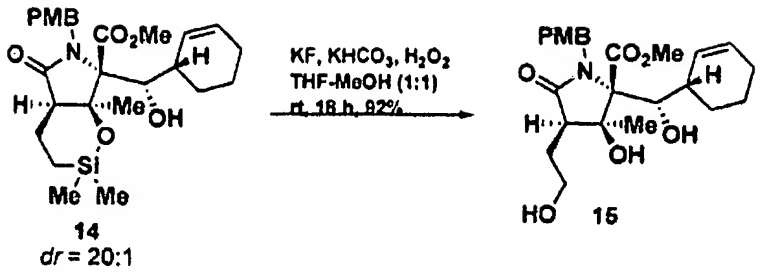
The next step, the attachment of the 2-cyclohexenly group to the formyl carbon and the establishment of the remaining two stereocenters was accomplished in a remarkably simple way.

2-Cyclohexenyl-tri-*n*-butyltin (from Pd(O)-catalyzed 1,4-addition of tributyltin hybride to 1,3-cyclohexadiene) was sequentially transmetalated by treatment with 1 equiv of *n*-butyllithium and 1 equiv of zinc chloride to form 2-cyclohexenylzinc chloride in THF solution. See, Miyake, H., Yamamura, K., *Chem. Lett.*, 1992, 507-508. Reaction of this reagent with the aldehyde **13** furnished the desired formyl adduct **14** stereoselectively (20:1) in 88% yield.



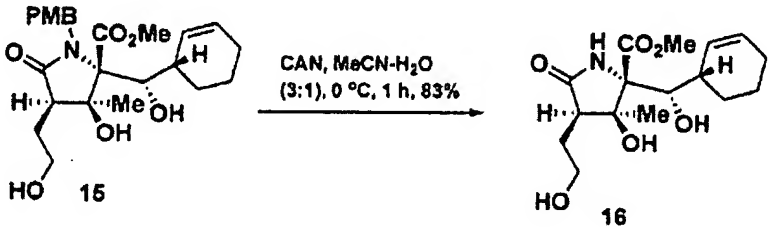
13 14
dr = 20:1

Tamao-Fleming oxidation of **14** gave the triol **15** in 92% yield.



See, Fleming, T., *Chemtracts-Org. Chem.,* 1996, 9, 1-64, and Jones, G. R., Landais, Y., *Tetrahedron,* 1996, 52, 7599-7662.

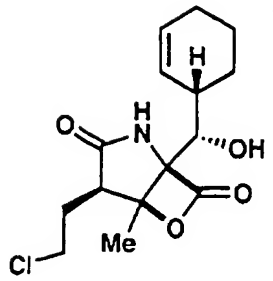
Ce(IV)-induced oxidative cleavage of the PMB group of **15** afforded the triol ester **16**:



Compound **16** was then hydrolyzed to the corresponding γ-lactam-carboxylic acid **16a** (CO_2Me is CO_2H) using 3:1 aqueous 3N-lithium hydroxide and THF at 4°C.

The acid **16a** was first cyclized to the beta-lactone **16b** (**1** where CH_2CH_2Cl is CH_2CH_2OH), which is then converted to salinosporamide A (**1**) by successive reaction with 1.1 equiv of *bis* (2-oxo-3-oxazolidinyl) phosphinic chloride (BOPCl) and pyridine at 23°C for 1 h, in 65% overall yield.

16a → 16b → 1



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The identity of synthetic **1** and natural Salinosporamide A was established by comparison measurements of ^{1}H and ^{13}C NMR spectra, mp and mixed mp (168-170°C), optical rotation, FTIR and mass spectra and chromatographic mobilities in three different solvent systems. Dr. Fenical graciously provided a sample of the natural product for this comparison.

There are a number of steps in the synthesis of **1** that require comment. The direct conversion of **6** to **7** with acrylyl chloride under a wide variety of conditions gave considerably lower yields than the process shown in Scheme 1 mainly because of competing O-acylation and subsequent further transformations.

The critical cyclization of **8** to **10** is still under investigation to find the conditions for maximizing the formation of **9** over the diastereomer **10.** So far, quinuclidine has proved superior to other catalytic bases tried, e.g., 1,4-diaza[2.2.2]bicycloocate. As indicated above, the attachment of the 2-cyclohexenyl group to aldehyde 13 to form 14 worked best with the reagent cyclohexenylzinc chloride.

The stereochemistry of the conversion **13** to **14**, established by the identity of totally synthetic **1** with naturally formed salinosporamide A, is that predicted from a cyclic, chair-formed, six-membered transition state involving addition of the organozinc reagent to the sterically more accessible face of the formyl group. The use of 2-cyclo-hexenylzinc chloride may be critical to successful formation of **14** since none of this

product is obtained with 2-cyclohexenyllithium (probably because the initial carbonyl adduct undergoes retroaldol cleavage and decomposition; see Corey, E. J., Li, W., Nagamitsu, T., *Angew. Chem. Int, Ed.*, 1998, 37, 1676-1679).

Attempts to form **14** from **13** using Lewis acid-catalyzed reaction with tri-*n*-butyl-2-cyclohexenyltin have thus far been unsuccessful. The saponification of methyl ester **16** at temperatures above +5°C led to lowered yields of the required carboxylic acid. Finally, the one flask β-lactonization and chlorination reactions leading to **1** were remarkably clean and probably proceed in greater than 90% yield per step.

In addition to the method of Scheme I, preferred embodiments of the invention also include novel synthetic intermediate compounds, intermediate steps of the preferred synthetic process; and the uses of this method and/or intermediate compounds thereof, in the preparation of synthetic analogs or derivatives of the compound Salinosporamide A. Typical substituent modifications for compounds of this type are known to persons having ordinary skill in this art. See, for example, the substituent groups defined for analogs of lactacystin compounds as taught in Corey et al., *Chem. Pharm. Bull.*, 1999, 47, 1-10, the disclosure of which is incorporated herein by reference. Other substituent modifications will be apparent based upon the disclosures in related patents. See, for example, U.S. Patent Nos. 6,645,999; 6,566,553; 6,458,825; 6,335,358; 6,294,560; 6,214,862; 6,147,223; 6,133,308; 5,869,675; 5,756,764; and PCT Publication No. WO 96/32105; the disclosures of which are hereby incorporated herein by reference.

BRIEF DESCRIPTION OF THE DRAWING

The Figure illustrates Scheme I, a preferred synthetic route used to achieve the enantiospecific total synthesis of the compound of structure **1**.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

As set forth above, one embodiment of the present invention comprises a simple and effective stereocontrolled synthesis of Salinosporamide A, the compound of formula (**1**). Scheme 1, shown in the Figure, is a preferred pathway to accomplish this synthesis, the details of which are provided in the following Examples.

Experimental Details

Part I. Synthesis of the Salinosporamide A

Part 2. Synthesis of the Cyclohexenyl zinc chloride

General.

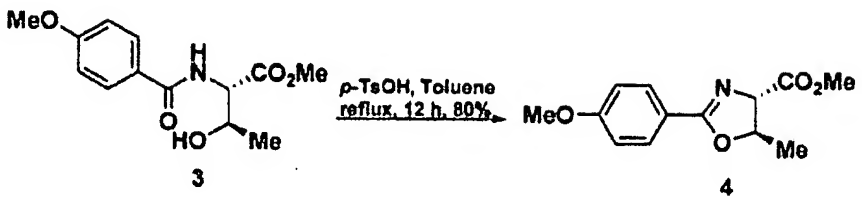
All moisture sensitive reactions were performed under nitrogen gas in glassware that was flame-dried and equipped with a magnetic stir bar. Tetrahydrofuran (THF) and 1,2-dimethoxyethane (DME) were freshly distilled from sodium benzophenone ketyl before use. Hexanes, pyridine, triethylamine, pentane and dichloromethane were freshly distilled from CaH_2 before use. Toluene was distilled from sodium.

Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F_{254} pre-coated plates (0.25 mm). Flash chromatography was performed using Baker silica gel (40 μm particle size). All products were purified to homogeneity by TLC analysis (single spot/two solvent systems) using a UV lamp or CAM or PMA or anisaldehyde or basic $KMnO_4$ for detection purposes.

NMR spectra were recorded on 400 MHz, 500 MHz and 600 MHz spectrometers. ^1H and ^{13}C NMR chemical shifts are reported as δ using residual solvent as an internal standard. High-resolution mass spectral analyses were performed at Harvard University Mass Spectrometry Center.

Part I. Synthesis of the Salinosporamide A (1)

Example 1:

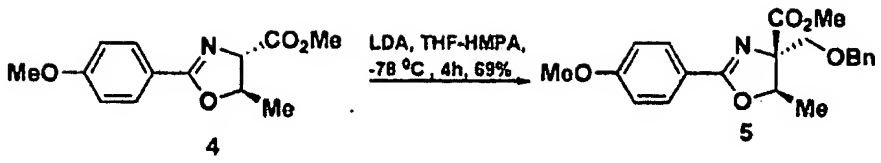


(4S, 5R) Methyl 4,5-dihydro-2 (4-methoxyphenyl)-5-methyloxazole-4-carboxylate (**4**).

A mixture of (2S, 3R)-methyl 2-(4-methoxybenzamido)-3-hydroxybutanoate (**3**) (35.0 g, 131 mmol) and p-TsOH·H_20 (2.5 g, 13.1 mmol) in toluene (400 mL) was heated at reflux for 12 h. The reaction mixture was diluted with water (200 mL) and extracted with EtOAc (3X 200 mL). The combined organic layers were washed with water, brine and dried over Na_2SO_4. The solvent was removed *in vacuo* to give crude oxazoline as yellow oil. Flash column chromatography on silica gel (eluent 15% EtOAc-Hexanes) afforded the pure oxazoline (26.1 g, 80%) as solid.

R_f = 0.51 (50% ethyl acetate in hexanes), mp, 86-87°C; $[\alpha]^{23}_D$ +69.4 (c 2.0, $CHC1_3$); FTIR (film) ν_{max}: 2955, 1750, 1545, 1355, 1187, 1011, 810 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.87 (2H, d, *J* = 9.2 Hz), 6.84 (2H, d, *J* = 8.8 Hz), 4.90 (1 H, m), 4.40 (1 H, d, *J* = 7.6 Hz), 3.79 (3H,s), 3.71 (3H, s), 1.49 (3H, d, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.93, 165.54, 162.64, 130.52, 119.80, 113.85, 78.91, 75.16, 55.51, 52.73, 21.14; HRMS (ESI) calcd for $C_{13}H_{16}NO_4$ $(M + H)^+$.250.1079, found 250.1084.

Example 2:

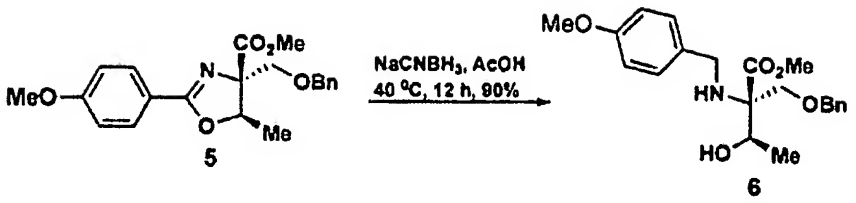


(4R, 5R)-Methyl 4-{(benzyloxy) methyl)}-4,5-dihydro-2- (4-methoxyphenyl)-5- methyloxazole-4-carboxylate (**5**).

To a solution of LDA (50 mmol, 1.0 M stock solution in THF) was added HMPA (24 mL, 215 mmol) at -78°C and then oxazoline **4** (12.45 g, 50 mmol, in 20 mL THF) was added dropwise with stirring at -78°C for 1 h to allow complete enolate formation. Benzyloxy chloromethyl ether (8.35 mL, 60 mmol) was added at this temperature and after stirring the mixture at -78°C for 4 h, it was quenched with water (50 mL) and warmed to 23°C for 30 min. Then the mixture was extracted with ethyl acetate (3 x 50 mL) and the combined organic phases were dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexanes, 1:4 then 1:3) to give the benzyl ether **5** (12.7 g, 69%).

R_f= 0.59 (50% ethyl acetate in hexanes). $[\alpha]^{23}_D$ -6.3 (c 1.0, $CHCl_3$); FTIR (film) (ν_{max}: 3050, 2975, 1724, 1642, 1607, 1252, 1027, 745, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.96 (2H, d, J= 9.2 Hz), 7.26 (5H, m), 6.90 (2H, J= 8.8 Hz), 4.80 (1 H,m), 4.61 (2H,s), 3.87 (3H, m), 3.81 (3H, s), 3.73 (3H, s), 1.34 (3H, d, J= 6.8 Hz); ^{13}C NMR ($CDCl_3$, 100MHz) : δ 171.23, 165.47, 162.63, 138.25, 130.64, 128.52, 127.87, 127.77, 120.15, 113.87, 81.40, 79.92, 73.91, 73.43, 55.58, 52.45, 16.92; HRMS (ESI) calcd for $C_{21}H_{24}O_5$ $(M + H)^+$ 370.1654, found 370.1644.

Example 3:

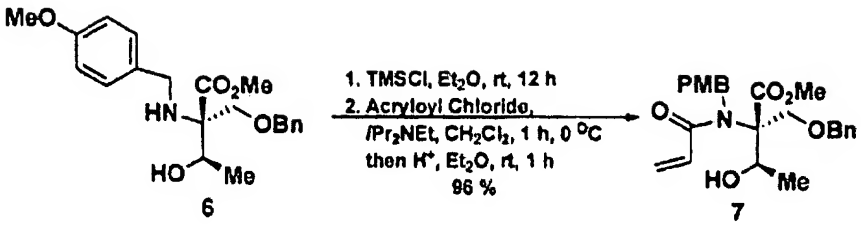


(2R,3R)-Methyl 2-(4-methoxybenzylamino)-2-((benzyloxy)methyl)-3hydroxybutanoate (**6**).

To a solution of oxazoline 5 (18.45 g, 50 mmol) in AcOH (25 mL) at 23°C was added in portions $NaCNBH_3$ (9.3 g, 150 mmol). The reaction mixture was then stirred at 40°C for 12 h to allow complete consumption of the starting material. The reaction mixture was diluted with water (100 mL), neutralized with solid Na_2CO_3 and the aqueous layer was extracted with ethyl acetate (3 x 100 mL). The combined organic phases were dried over $NaSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexanes, 1:5) to give the *N*-PMB amino alcohol **6** (16.78 g, 90%).

R_f = 0.50 (50% ethyl acetate in hexanes). $[\alpha]23_D$ -9.1(c 1.0, $CHCl_3$); FTIR (film) ν_{max}: 3354, 2949, 1731, 1511, 1242, 1070, 1030, 820,736, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.32 (7H, m), 6.87 (2H, d, *J* = 8.8 Hz), 4.55 (2H, m), 4.10 (1 H, q, *J* = 6.4 Hz), 3.85 (2H, dd, *J* = 17.2, 10.0 Hz), 3.81 (3H, s,), 3.77 (3H, s), 3. 69 (2H, dd, *J* = 22.8, 11.6 Hz), 3.22 (2H, bs), 1.16 (3H, d, *J* = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.34, 159.03, 137.92, 132.51, 129.78, 128.67, 128.07, 127.98, 114.07, 73.80, 70.55, 69.82, 69.65, 55.51, 55.29, 47.68, 18.15; HRMS (ESI) calcd. for $C_{21}H_{28}NO_5$ $(M + H)^+$ 374.1967, found 374.1974.

Example 4:

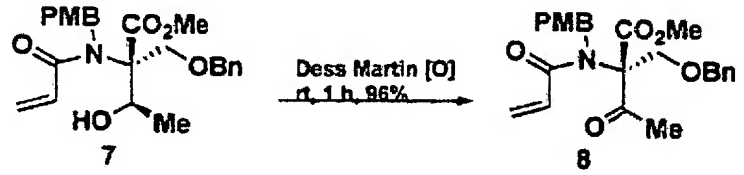


(2R,3R)-Methyl-2-(N-(4-methoxybenzyl)acrylamido)-2-(benzyloxy)methyl)-3-hydroxybutanoate (**7**).

A solution of amino alcohol **6** (26.2 g, 68.5 mmol) in Et_2O (200 mL) was treated with Et_3N (14.2 mL, 102.8 mmol) and trimethylchlorosilane (10.4 mL, 82.2 mmol) at 23°C and stirred for 12 h. After completion, the reaction mixture was diluted with ether (200 mL) and then resulting suspension was filtered through celite. The solvent was removed to furnish the crude product (31.2 g, 99%) in quantitative yield as viscous oil. A solution of this crude trimethylsilyl ether (31.1 g) in CH_2Cl_2 (200 mL) was charged with diisopropylethylamine (14.2 mL, 81.6 mmol) and then cooled to 0°C. Acryloyl chloride (6.64 mL, 82.2 mmol) was added dropwise with vigorous stirring and the reaction temperature was maintained at 0°C until completion (1 h). The reaction mixture was then diluted with CH_2Cl_2 (100 mL) and the organic layer was washed with water and brine. The organic layer was separated and dried over Na_2SO_4. The solvent was removed to afford the crude acrylamide **7** as a viscous oil. The crude product was then dissolved in Et_2O (200 mL) and stirred with 6N HCl (40 mL) at 23°C for 1 h. The reaction mixture was diluted with water (100 mL) and concentrated to provide crude product. The residue was purified by column chromatography (silica gel, ethyl acetate/hexanes, 1:5 to 1:1) to give pure amide **7** (28.3 g, 96%) as colorless solid, mp 88-89°C.

R_f= 0.40 (50% ethyl acetate in hexanes), $[\alpha]^{23}_D$ -31.1 (c 0.45, $CHCl_3$), FTIR (film) ν_{max}: 3435, 2990, 1725, 1649, 1610, 1512, 1415, 1287, 1242, 1175, 1087, 1029, 732, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.25 (5H, m), 7.15 (2H, d, J = 6.0 Hz), 6.85 (2H, d, J = 7.5 Hz), 6.38 (2H, d, J = 6.0 Hz), 5.55 (1 H, t, J = 6.0 Hz), 4. 81 (2H, s), 4.71 (1 H, q, J = 6.5 Hz), 4.35 (2H, s), 4.00 (1 H, d, J = 10.0 Hz), 3.80 (1 H, d, J = 10.0 Hz), 3.76 (3H, s), 3.75 (3H, s), 3.28 (1 H, bs), 1.22 (3H, d, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.87, 168.74, 158.81, 137.73, 131.04, 129.68, 128.58, 128.51, 127.94, 127.72, 127.20, 127.14, 114.21, 73.71, 70.42, 69.76, 67.65, 55.45, 52.52, 49.09, 18.88; HRMS (ESI) calcd. for $C_{24}H_{30}NO_6$ $(M + H)^+$428.2073, found 428.2073.

Example 5:



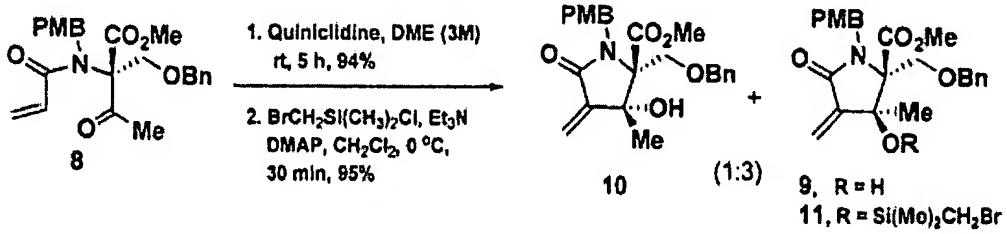
(R)-Methyl-2-(N-(4-methoxybenzyl)acrylamido)-2-(benzyloxy)methyl)-3-oxybutanoate (**8**).

To a solution of amide **7** (10.67 g, 25.0 mmol) in CH_2Cl_2 (100 mL) was added Dess-Martin periodinane reagent (12.75 g, 30.0 mmol, Aldrich Co.) at 23°C. After stirring for 1 h, the reaction mixture was quenched with aq $NaHCO_3$-$Na_2S_2O_3$ (1:1, 50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic phase was dried and concentrated *in vacuo* to afford the crude ketone. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexanes) to give pure keto amide **8** (10.2 g, 96%).

R_f= 0.80 (50% ethyl acetate in hexanes), mp 85 to 86°C; $[\alpha]^{23}_D$ -12.8 (c 1.45, $CHCl_3$); FTIR (film) ν_{max}: 3030, 2995, 1733, 1717, 1510, 1256, 1178, 1088, 1027, 733, 697 cm^{-1};

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.30 (2H, d, J = 8.0), 7.25 (3H, m), 7.11 (2H, m), 6.88 (2H, d, J = 9.0 Hz), 6.38 (2H, m), 5.63 (1 H, dd, J = 8.5, 3.5 Hz), 4. 93 (1 H, d, J = 18.5 Hz), 4.78 (1 H, d, J = 18.5, Hz), 4.27 (2H, m), 3.78 (3H, s), 3.76 (3H, s), 2.42 (3 H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 198.12, 169.23, 168.62, 158.01, 136.95, 130.64, 130.38, 128.63, 128.13, 127.77, 127.32, 114.33, 77.49, 73.97, 70.66, 55.49, 53.09, 49.03, 28.24; HRMS (ESI) calcd. for $C_{24}H_{28}NO_6$ $(M + H)^+$ 426.1916, found 426.1909.

Example 6:



(2R,3S)-Methyl-1-(4-methoxybenzyl)-2-((benzyloxy)methyl)-3-hydroxy-3-methyl-4-methylene-5-oxopyrrolidine-2-carboxylate (**9** + **10**).

A mixture of keto amide **8** (8.5 g, 20.0 mmol) and quinuclidine (2.22 g, 20.0 mmol) in DME (10 mL) was stirred for 5 h at 23°C. After completion, the reaction mixture was diluted with ethyl acetate (50 mL) washed with 2N HCl, followed by water and dried over Na_2SO_4. The solvent was removed *in vacuo* to give the crude adduct (8.03 g, 94.5%, 3:1 ratio of **9** to **10** *dr*) as a viscous oil. The diastereomeric mixture was separated at the next step, although small amounts of **9** and **10** were purified by column chromatography (silica gel, ethyl acetate/hexanes, 1:10 to 1:2) for analytical purposes.

Major Diastereomer (**9**).

$[\alpha]^{23}_D$ -37.8 (c 0.51, $CHCl_3$); FTIR (film) ν_{max}: 3450, 3055, 2990, 1733, 1683, 1507, 1107, 1028, 808,734 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.29 (5H, m), 7.15 (2H, d, J = 7.5 Hz), 6.74 (2H, d, J = 8.5 Hz), 6.13 (1 H, s), 5.57 (1 H,s), 4.81 (1 H, d, J = 14.5 Hz), 4.45(1 H, d, J = 15.0 Hz), 4.20 (1 H, d, J = 12.0 Hz), 4.10 (1 H, d, J = 12.0 Hz) 3.75 (3H, s), 3.70 (1 H, d, J = 10.5 Hz), 3.64 (3H, s), 3.54 (1 H, d, J = 10.5 Hz), 2.55 (1 H, bs, OH), 1.50 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.67, 168.42, 158.97, 145.96, 137.57, 130.19, 130.12, 128.53, 127.83, 127.44, 116.79, 113.71, 76.32, 76.00, 73.16, 68.29, 55.45, 52.63, 45.36, 22.64; HRMS (ESI) calcd. for $C_{24}H_{28}NO_6$ $(M + H)^+$ 426.1916, found 426.1915.

Minor Diastereomer (**10**).

$[\alpha]^{23}_D$ -50.1 (c 0.40, $CHCl_3$); FTIR (film) ν_{max}: 3450, 3055, 2990, 1733, 1683, 1507, 1107, 1028, 808, 734 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.29 (5H, m), 7.12 (2H, d, J = 7.5 Hz), 6.73 (2H, d, J = 8.5 Hz), 6.12 (1 H, s), 5.57 (1 H, s), 4.88 (1 H, d, J = 15.5 Hz), 4.31 (1 H, d, J = 15.0 Hz), 4.08 (3H, m), 3.99 (1 H, d, J = 12.0 Hz) 3.73 (3H, s), 3.62 (3H, s), 3.47 (1 H, bs, OH), 3.43 (1 H, d, J = 10.0 Hz), 1.31 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.65, 167.89, 159.13, 147.19, 136.95, 130.29, 129.76, 128.74, 128.19,127.55, 116.80, 113.82, 76.21, 75.66, 73.27, 68.02, 55.45, 52.52, 45.24, 25.25; HRMS (ESI) calcd. for $(M + H)^+$ $C_{24}H_{28}NO_6$ 426.1916, found 426.1915.

Example 7:

Silylation of **9** and **10** and Purification of **11**.

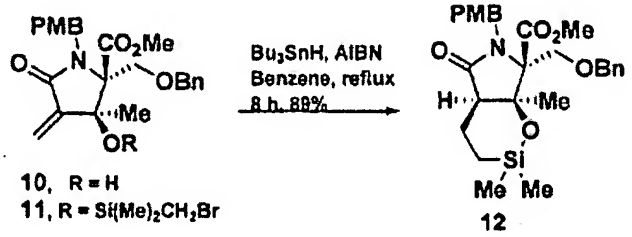
To a solution of lactams **9** and **10** (7.67 g, 18 mmol) in CH_2Cl_2 (25 ml) was added Et_3N (7.54 ml, 54 mmol), and DMAP (2.2 g, 18 mmol) at 0°C, and then bromomethyl-dimethylchlorosilane (5.05 g, 27 mmol) (added dropwise). After stirring the mixture for

30 min at 0°C, it was quenched with aq $NaHCO_3$ and the resulting mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were washed with water, brine and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a mixture of the silated derivatives of **9** and **10** (9.83 g, 95%). The diastereomers were purified by column chromatography (silica gel, ethyl acetate/hexanes, 1:5 to 1:4) to give pure diastereomer **11** (7.4 g, 72%) and its diastereomer (2.4 g, 22%).

Silyl Ether (**11**).

R_f= 0.80 (30% ethyl acetate in hexanes). $[\alpha]^{23}_D$ -58.9 (c 0.55, $CHCl_3$); FTIR (film) ν_{max}: 3050, 2995, 1738, 1697, 1512, 1405, 1243, 1108, 1003, 809, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.27 (5H, m), 7.05 (2H, d, J = 7.0 Hz), 6.71 (2H, d, J = 8.5 Hz), 6.18 (1 H, s), 5.53 (1 H,s), 4.95 (1 H, d, J = 15.5 Hz), 4.45 (1 H, d, J = 15.0 Hz), 4.02 (1 H, J = 12.0 Hz), 3.86 (1 H, d, J = 11.5 Hz) 3.72 (3H, s), 3.68 (3H, s), 3. 65 (1 H, d, J = 10.5 Hz), 3.30 (1 H, d, J = 10.0 Hz), 2.34 (2H, d, J = 2.0 Hz), 1.58 (3H, s), 0.19 (3 H, s), 0.18 (3 H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 168.62, 168.12, 158.93, 145.24, 137.53, 130.32, 130.30, 128.49, 127.76,127.22, 117.26, 113.60, 78.55, 78.03, 72.89, 68.45, 55.43, 52.37, 45.74, 21.87, 17.32, -0.72, -0.80; HRMS (ESI) Calcd. for $C_{27}H_{35}BrNO_6Si$ $(M + H)^+$ 576.1417, found 576.1407.

Example 8:

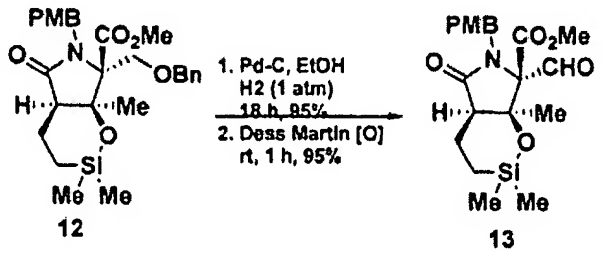


Conversion of (**11**) to (**12**).

To a solution of compound **11** (5.67 g 10 mmol) in benzene (250 mL) at 80°C under nitrogen was added a mixture of tributyltin hydride (4.03 ml, 15 mmol) and AIBN (164 mg, 1 mmol) in 50 ml benzene by syringe pump over 4 h. After the addition was complete, the reaction mixture was stirred for an additional 4 h at 80°C and the solvent was removed *in vacuo*. The residue was dissolved in hexanes (20 mL) and washed with saturated $NaHCO_3$ (3 x 25 mL), water and dried over Na_2SO_4. The solvent was removed *in vacuo* to give crude product. The crude product was purified by column chromatography (silica gel, ethyl acetate/hexanes, 1: 5) to afford the pure **12** (4.42 g, 89%).

R_f= 0.80 (30% ethyl acetate in hexanes). $[\alpha]^{23}_D$ -38.8 (c 0.25, $CHCl_3$); FTIR (film) ν_{max}: 3025, 2985, 1756, 1692, 1513, 1247, 1177, 1059, 667 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.28 (5H, m), 7.09 (2H, d, *J* = 7.0 Hz), 6.73 (2H, d, *J* = 9.0 Hz), 4.96(1H,d, *J*=15.0Hz), 4.35 (1H,d, *J*=15.5Hz), 3.97 (1H, d, *J*=12.5Hz), 3.86 (1H, d, *J* = 12.0 Hz), 3.80 (1 H, d, *J* = 10.0 Hz), 3.72 (3H, s), 3.65 (3H, s), 3.27 (1 H, d, *J* = 10.5 Hz), 2.67 (1 H, t, *J* = 4.0 Hz), 2.41 (1 H, m), 1.79 (1 H, m), 1.46 (3H, s), 0.77 (1 H, m), 0.46 (1H, m), 0.10 (3H, s), 0.19 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.48, 169.46, 158.76, 137.59, 131.04, 129.90, 128.58, 127.88,127.52, 113.59, 113.60, 81.05, 78.88, 73.12, 69.03, 55.45, 51.94, 48.81, 45.50, 22.79, 17.06, 7.76, 0.54; HRMS (ESI) calcd. for $(M + H)^+$ $C_{27}H_{36}NO_6Si$ 498.2312, found 498.2309.

Example 9:



Debenzylation of (**12**).

A solution of **12** (3.98 g, 8 mmol) in EtOH (50 ml) at 23°C was treated with 10% Pd-C (~1 g) under an argon atmosphere. The reaction mixture was evacuated and flushed with H_2 gas (four times) and then stirred vigorously under an atmosphere of H_2 (1 atm, H_2 balloon) at 23°C. After 12 h, the reaction mixture was filtered through Celite and concentrated *in vacuo* to give the crude debenzylation product (3.08 g, 95%) which was used for the next step. A small amount crude product was purified by column chromatography (silica gel, ethyl acetate/hexanes, 1: 3) for analytical purposes. R_f= 0.41 (50% ethyl acetate in hexanes).

mp, 45 - 47°C; $[\alpha]^{23}_D$ -30.9 (c 0.55, $CHCl_3$); FTIR (film) ν_{max}: 3432, 3020, 2926, 1735, 1692, 1512, 1244, 1174, 1094, 1024, 870, 795 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.36 (2H, d, *J* = 8.5 Hz), 6.83 (2H, d, *J* = 8.5 Hz), 5.16 (1 H, d, *J* = 15.0 Hz), 4.29 (1 H, d, *J* = 15.0 Hz), 3.92 (1 H, m), 3.78 (3H, s), 3.68 (3H, s), 3.45 (1 H, m), 2.53 (1 H, t, *J* = 4.0 Hz), 2.42 (1 H, m), 1.82 (1 H, m), 1.50 (3H, s), 1.28 (1 H, m), 0.75 (1 H, m), 0.47 (1 H, m), 0.11 (3H, s), 0.02 (3H, s) ; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 175.82, 169.51, 159.32, 131.00, 129.72, 114.52, 80.79, 80.13, 61.85, 55.48, 51.99, 49.29, 45.06, 23.11, 17.03, 7.44, 0.54; HRMS (ESI) calcd. for $C_{20}H_{30}N0_6Si$ $(M + H)^+$ 408.1842, found 408.1846.

Example 10:

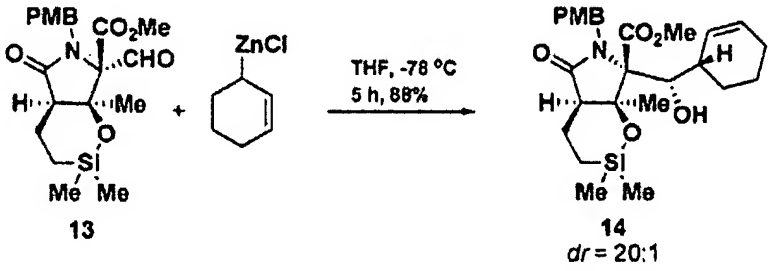
Oxidation to Form Aldehyde (**13**).

To a solution of the above alcohol from debenzylation of **12** (2.84 g, 7 mmol) in CH_2Cl_2 (30 mL) was added Dess-Martin reagent (3.57 g, 8.4 mmol) at 23°C. After stirring for 1 h at 23°C, the reaction mixture was quenched with aq $NaHCO_3$-$Na_2S_2O_3$ (1:1, 50 mL) and extracted with ethyl acetate (3 x 50 mL). The organic phase was dried and concentrated *in vacuo* to afford the crude aldehyde. The crude product was purified

by column chromatography (silica gel, ethyl acetate/hexanes, 1:5) to give pure aldehyde **13** (2.68 g, 95%). R_f = 0.56 (50% ethyl acetate in hexanes).

mp, 54-56°C; $[\alpha]^{23}_D$ -16.5 (c 0.60, $CHCl_3$); FTIR (film) ν_{max}: 3015, 2925, 1724, 1702, 1297, 1247, 1170, 1096, 987, 794 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 9.62 (1 H, s), 7.07 (2H, d, *J* = 8.0 Hz), 6.73 (2H, d, *J* = 8.5 Hz), 4.49 (1 H, quart, *J* = 8.5 Hz), 3.70 (3H, s), 3.67 (3H, s), 2.36 (2H, m), 1.75 (1H, m), 1.37 (3H, s), 0.73 (1 H, m), 0.48 (1 H, m), 0.07 (3H, s), 0.004 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 197.26, 174.70, 167.36, 158.07, 130.49, 128.96, 113.81, 83.97, 82.36, 55.34, 52.43, 47.74, 46.32, 23.83, 16.90, 7.52, 0.56, 0.45; HRMS (ESI) calcd. for $C_{20}H_{28}NO_6Si$ $(M + H)^+$ 406.1686, found 406.1692.

Example 11:



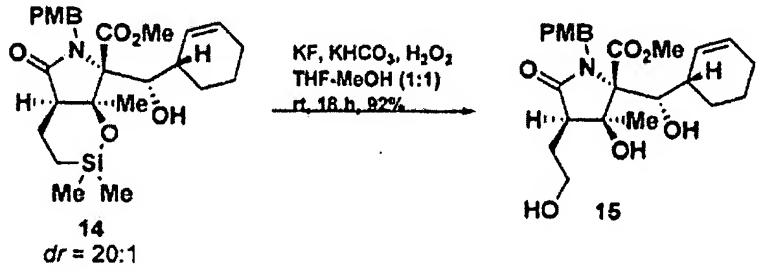
Conversion of (**13**) to (**14**).

To a solution of freshly prepared cyclohexenyl zinc chloride (10 mL, 0.5 M solution in THF, 5 mmol) (see Example 15 below) at -78°C under nitrogen was added a -78°C solution of aldehyde **13** (1.01 g, in 3 ml of THF, 2.5 mmol). After stirring for 5 h at -78°C reaction mixture was quenched with water (10 mL) then extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and solvent was removed *in vacuo* to give crude product (20 : 1 *dr*). The diastereomers were purified by column chromatography (silica gel, ethyl acetate/hexanes, 1:10 to 1:2 affords the pure

major diastereomer **14** (1.0 g, 83%) and a minor diastereomer (50 mg 5%). For **14**: R_f= 0.56 (50% ethyl acetate in hexanes).

mp, 79-81°C; $[\alpha]^{23}_D$ -28.5 (*c* 1.45, $CHCl_3$); FTIR (film) ν_{max}: 3267, 2927, 2894, 2829, 1742, 1667, 1509, 1248, 1164, 1024, 795 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.34 (2H, d, *J* = 8.5 Hz), 6.81 (2H, d, *J* = 9.0 Hz), 5.84 (1 H, m), 5.73 (1 H, m), 4.88 (1 H, d, *J* = 15.5 Hz), 4.39 (1 H, d, *J* = 14.5 Hz), 4.11 (1 H, t, *J* = 6.5 Hz), 3.77 (3H, s), 3.58 (3H, s), 3.00 (1 H, m), 2.95 (1 H, d, *J* = 9.0 Hz), 2.83 (1 H, t, *J* = 3.5 Hz), 3.36 (1 H, m), 2.27 (1H, m), 1.98 (2H, m), 1.74 (3H, m), 1.62 (3H, s), 1.14 (2H, m), 0.59 (1H, m), 0.39 (11H, m), 0.13 (3H, s), 0.03 (3H, s) ; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.80, 170.03, 158.27, 131.86, 131.34, 128.50, 126.15, 113.40, 83.96, 82.45, 77.17, 55.45, 51.46, 48.34, 48.29, 39.08, 28.34, 25.29, 22.45, 21.09, 17.30, 7.75, 0.39, 0.28; HRMS (ESI) calcd. for $C_{26}H_{38}NO_6Si$ $(M + H)^+$ 488.2468, found 488.2477.

Example 12:



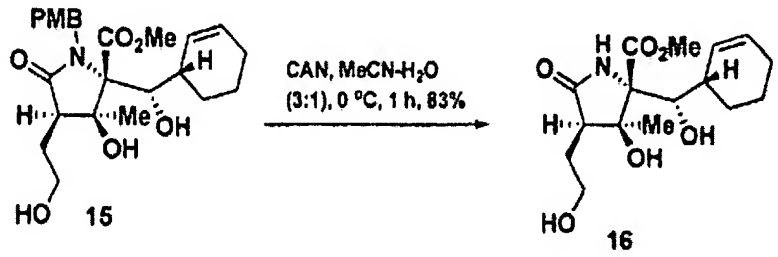
Tamao-Fleming Oxidation of (**14**) to (**15**).

To a solution of **14** (0.974 g, 2 mmol) in THF (5 mL) and MeOH (5 mL) at 23°C was added $KHCO_3$ (0.8 g, 8 mmol) and KF (0.348 g, 6 mmol). Hydrogen peroxide (30% in water, 5 mL) was then introduced to this mixture. The reaction mixture was vigorously stirred at 23°C and additional hydrogen peroxide (2 ml) was added after 12 h. After 18h, the reaction mixture was quenched carefully with $NaHSO_3$ solution (15 mL).

The mixture was extracted with ethyl acetate (3 x 25 mL) and the combined organic layers were washed with water and dried over Na_2SO_4. The solvent was removed *in vacuo* to give the crude product. The crude product was purified by column chromatography (silica gel, ethyl acetate) to give the pure triol **15** (0.82 g, 92%).

R_f = 0.15 (in ethyl acetate). mp, 83-84°C; $[\alpha]^{23}_D$: +5.2 (*c* 0.60, $CHC1_3$); FTIR (film) ν_{max}: 3317, 2920, 2827, 1741, 1654, 1502, 1246, 1170, 1018, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.77 (2H, d, *J* = 8.0 Hz), 6.28 (2H, d, *J* = 8.0 Hz), 5. 76 (1 H, m), 5.63 (1H, d, *J* = 10.0 Hz), 4.74 (1H, d, *J* = 15.5 Hz), 4.54 (1H, d, *J* = 15.0 Hz), 4.12 (1H, d, , *J* = 2.5 Hz), 3.80 (1 H, m), 3.76 (3H, s), 3.72 (1 H, m), 3.68 (3H, s), 3.00 (1 H, m), 2.60 (1 H, br), 2.20 (1 H, m), 1.98 (2H, s), 1.87 (1 H, m), 1.80 (1 H, m), 1.71 (2H, m), 1.61 (3 H, s), 1.14 (2 H, m); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 178.99, 170.12, 158.27, 131.30, 130.55, 128.13, 126.39, 113.74, 81.93, 80.75, 76.87, 61.61, 55.45, 51.97, 51.32, 48.07, 39.17, 27.71, 27.13, 25.22, 21.35, 21.22; HRMS (ESI) calcd. for $C_{24}H_{34}NO_7$ $(M + H)^+$ 448.2335, found 448.2334.

Example 13:



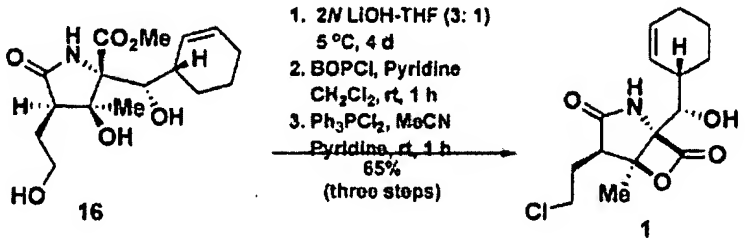
Deprotection of (**15**) to (**16**).

To a solution of **15** (0.670 g, 1.5 mmol) in acetonitrile (8 mL) at 0°C was added a pre-cooled solution of ceric ammonium nitrate (CAN) (2.46 g 4.5 mmol in 2 mL H_2O). After stirring for 1 h at 0°C the reaction mixture was diluted with ethyl acetate (50 mL),

washed with saturated NaCl solution (5 mL) and organic layers was dried over Na_2SO_4. The solvent was removed *in vacuo* to give the crude product which was purified by column chromatography (silica gel, ethyl acetate) to give the pure **16** (0.4 g, 83%).

R_f = 0.10 (5% MeOH in ethyl acetate). mp, 138 to 140°C; $[\alpha]^{23}_D$ +14.5 (*c* 1.05, $CHCl_3$); FTIR (film) ν_{max} 3301, 2949, 2911, 2850, 1723, 1673, 1437, 1371, 1239, 1156, 1008, 689 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz): δ 8.48 (1H, br), 6.08 (1H, m), 5. 75 (1 H, d, *J* = 9.6 Hz), 5.29 (1 H, br), 4.13 (1 H, d, *J* = 6.6 Hz), 3.83 (3H, m), 3.79 (1 H, m), 3.72 (1 H, m), 2.84 (1 H, d, *J* = 10.2 Hz), 2.20 (1 H, m), 2.16 (1 H, br), 1.98 (3H, m), 1.77 (3H, m), 1.59 (1 H, m), 1.54 (3H, s), 1.25 (1 H, m). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 180.84, 172.95, 135.27, 123.75, 82.00, 80.11, 75.56, 62.39, 53.14, 51.78, 38.95, 28.79, 26.48, 25.04, 20.66, 19.99; HRMS (ESI) calcd. $(M + H)^+$ for $C_{16}H_{26}NO_6$ 328.1760, found 328.1752.

Example 14:



Conversion of (**16**) to Salinosporamide A (**1**).

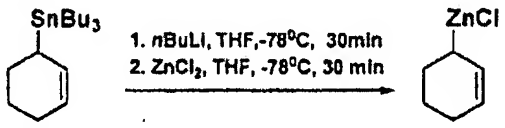
A solution of triol ester **16** (0.164 g, 0.5 mmol) in 3 N aq LiOH (3 mL) and THF (1 mL) was stirred at 5°C for 4 days until hydrolysis was complete. The acid reaction mixture was acidified with phosphoric acid (to pH 3.5). The solvent was removed *in vacuo* and the residue was extracted with EtOAc, separated, and concentrated *in vacuo* to give the crude trihydroxy carboxylic acid **16a** (not shown). The crude acid was suspended in dry CH_2C1_2 (2 mL), treated with pyridine (0.5 mL) and stirred vigorously at 23°C for 5 min. To this solution was added BOPCl (152 mg, 0.6 mmol) at 23°C under

argon, and stirring was continued for 1h. The solvent was removed under high vacuum and the residue was suspended in dry CH_3CN (1 mL) and treated with pyridine (1 mL). To this solution was added PPh_3Cl_2 (333 mg, 1.0 mmol) at 23°C under argon with stirring. After 1 h the solvent was removed *in vacuo*. The crude product was purified by column chromatography (silica gel, ethyl acetate-CH_2Cl_2, 1 : 5) to give the pure β - lactone **1** (100 mg, 64%) as a colorless solid.

R_f = 0.55 (50% ethyl acetate in hexane). mp, 168-170°C (authentic sample: 168-170°C, 169-171°C in *Angew. Chem. Int. Ed.*, 2003, 42, 355-357); mixture mp, 168-170°C. $[\alpha]^{23}_D$ -73.2 (*c* 0.49, MeOH) , -72.9 (*c* 0.55, MeOH, in *Angew. Chem. Int. Ed.*, 2003, 42, 355-357); FTIR (film) ν_{max}: 3406, 2955, 2920, 2844, 1823, 1701, 1257, 1076, 1012, 785, 691 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 10.62 (1 H, br), 6.42 (1 H, d, *J* = 10.5 Hz), 5. 88 (1 H, m), 4.25 (1 H, d, *J* = 9.0 Hz), 4.14 (1 H, m), 4.01 (1 H, m), 3.17 (1 H, t, *J* = 7.0 Hz), 2.85 (1 H, m), 2.48 (1 H, m), 2.32 (2H, m), 2.07 (3H, s), 1.91 (2H, m), 1.66 (2H, m), 1.38 (1 H, m); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 176.92, 169.43, 129.08, 128.69, 86.32, 80.35, 70.98, 46.18, 43.28, 39.31, 29.01, 26.47, 25.35, 21.73, 20.00; HRMS (ESI) calcd. for (M - H)$^-$ $C_{15}H_{19}ClNO_4$ 312.1003, found 312.1003.

Part 2. Synthesis of the 2-Cyclohexenylzinc chloride

Example 15:



Synthesis of the Cyclohexenylzinc chloride.

To a solution of cyclohexenyltributyl tin (1.85 g 5 mmol) in THF (5ml) at -78°C under nitrogen was added *n*BuLi (2 ml, 2.5M solution in hexane, 5 mmol). See Miyake,

H., Yamamura, K., *Chem. Lett.*, 1992, 507-508. After an additional 30 min stirring, $ZnCl_2$ (5 ml, 1 M solution in THF, 5 mmol) was added and stirring was continued at this temperature for 30 min at -78°C to give a 0.5M solution of 2-cyclohexenylzinc chloride for reaction with the aldehyde **13** (see p S12).

The present invention has been described in detail, including the preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the present disclosure, may make modifications and/or improvements on this invention and still be within the scope of this invention as set forth in the following claims.